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The Effects of CO_2-Enriched Water Irrigation on Agricultural Crop Growth

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Abstract: CO_2, a major industrial (waste)water treatment process byproduct, significantly contributes to climate change, desertification and overall water depletion. Therefore, there is a significant interest in decreasing CO_2 amounts, generated by various technological processes, through a wide range of methods from geological sequestration to biological sequestration. The CO_2 (waste)water treatment byproduct sequestration into agricultural CO_2-enhanced irrigation water offers several benefits by enhancing crop yield and repurposing emissions. This sustainable approach supports climate neutrality via biological sequestration, promotes circular economy principles, and strengthens the link between agriculture and climate change. In this study, the effect of CO_2-enriched water irrigation was analyzed in a complex network of plants germination, soil bacterial populations' dynamics and soil composition. Results showed that germination rates of plants irrigated with CO_2-enriched water were species specific. Sage plants increased their germination and growth when irrigated with CO_2-enriched water compared with plants irrigated with plain water. Moreover, CO_2 addition favored the development of soil anaerobic bacteria in detriment of aerobic bacteria and subsequently changing organic and nitrogenous compounds soil composition compared to plain water irrigation. For the first time, the germination process influenced by CO_2 was correlated with on overall possible CO_2 effects on bacterial population growth dynamics and soil quality metabolites availability.

Keywords: CO_2 effect; aerobic and anaerobic bacteria; plant growth; circular economy

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1. Introduction

Anthropogenic activities have been increasingly generating pollutants with a major negative effect on the environment. Greenhouse gases (GHG) such as carbon dioxide (CO_2) have already perturbed the Earth's energy budget and changed Earth's climate.

Unfortunately, there is projected to massively increase and to result in considerable climatic change over the next decades. Climate change has been directly linked to longer droughts periods on larger areas due to an accelerated water evaporation, which depleted water natural resources and, subsequently, crop production and sustainability [1]. Water resources preservation is one of major societal challenges; therefore, a wide range of solutions have been proposed from wastewater treatment to drinkable water production. These water sustainable solutions aim to respond to a constant increase in water demand while water resources are in a continuous decline. Biological wastewater treatment and drinkable water production procedures have generated a growing amount of CO_2 released into atmosphere, which creates a vicious circle between water sustainability and GHG production. Wastewater treatments, the most energy consuming processes in the water sector, have produced more than 50 Million tons of CO_2 per year in Europe alone. The European Union's Green Deal aims to reach by 2030 around 45% greenhouse gas emissions from 1995 levels [2]. Unfortunately, these technologies have a high carbon footprint that could enhance the greenhouse effect, climate change and environmental water depletion in a vicious circle [3]. Possible mitigation solutions to address the CO_2 production, as a bypass product from (waste)water treatment processes, could be CO_2 byproduct sequestration as carbonated water. This CO_2-enriched water could be valorized, according to circular economy principles, as irrigation water. Overall, this is a potential powerful mitigation solution based on CO_2 capture and further use, since carbon can be assimilated into biotic and abiotic components from the environment. Incorporation of the CO_2 into biomass enhanced the circular economy, especially in agriculture when cultivating plants with nutritional and economical value. Plants with high economical value, so called cash crops, could benefit from irrigation with carbonated water by boosting their production and, subsequently, increasing their profit. In addition, the biomass production of CO_2 could have an extra economical value through combustion or biodegradation, especially when an eco-friendly biogenic CO_2 was released. The biogenic CO_2 released, unless CO_2 released based from fossil fuel combustion, could be very quickly inserted in a new biomass production by photosynthesis, being a part of natural short carbon cycle [4]. Since the mid-19th century, researchers have studied plant carbon absorption. Early experiments found that oat plants were initially impaired by CO_2-enriched water but later they thrived, sparking the debate on root or leaf absorption [5]. Later studies kept being inconclusive, some of which suggested minimal root absorption [6]. Other studies reported no plant yield increase due to a negative CO_2 impact on soil [7]. Subsequent studies [8–10] found that CO_2 hinders roots growth, although other studies presented CO_2 assimilation [11], inclusive CO_2 atmospheric via *Stylites andicola* root-based [12]. CO_2 enhanced the biosynthesis of auxins, a plant growth hormone, by upregulating YUCCA genes involved in indole-3-acetic acid (IAA) synthesis, leading to increased auxin levels in *Arabidopsis thaliana* [13]. However, CO_2 injection above a concentration of 41.3% in soil gaseous volume has been reported to influence nutrient availability in the soil, negatively affecting roots development and plant growth [14]. In spite of the fact that there was not a direct report in the literature, CO_2 has been perceived to have a slow diffusion of CO_2 from soil into the boundary layer of topsoil, being heavier than air. CO_2 could create a topsoil fumigant-like zone with negative impact on pest like locust larva incubation and, therefore, a thriving zone for plants growth. Monitoring and adjusting soil CO_2 content and pH is, thus, crucial to ensure the optimal availability of nutrients required for germination and growth. While there is certain research on the effects of elevated soil CO_2 levels on plant parameters, there is a noticeable lack of studies that linked it with changes in soil microbial parameters as well as microbial enzyme activities. Some studies showed that an increased CO_2 levels altered soil microbial dynamics by affecting organic matter decomposition and nutrient cycling, thereby affecting soil health and ecosystem functioning [15–17].

In this research, the CO_2 impact on agriculture, especially on plant growth, was analyzed by using carbonated water, mimicking a possible reduction of carbon footprint generated by (waste)water treatment technological processes. The carbonated water was produced in vitro by injecting commercially available CO2 into tap water. The relationship between carbonated water irrigation and plant growth, bacterial populations dynamic in soil and soil physicochemical parameters and metabolites was studied. The CO_2 effect on *Lamiaceae* family plants was species-specific, having a positive effect on sage, but negative on rosemary when compared to control plants with plain water irrigation. Soil's parameters (such as pH, electrical conductivity, or humidity) were also influenced by CO_2 concentration, so they could play a role in enhancing the germination. In addition, seed germination under CO_2 effect was linked to a bacterial population change and, therefore, to different metabolites proportions, especially nitrogen compounds, in soils irrigated with carbonated water. An overall picture showed a relationship between CO_2 in the soil and the plant germination process through its impact on the surrounding environment, including soil composition and bacterial populations. In perspective, irrigation with carbonated water can be a synergistic route for re-using CO_2 emissions by carbon-intensive industries (e.g., steel, cement, or chemical ones) and improving the productivity of suitable crops. This study assessed whether a higher CO_2 amount could be bioaccumulated and, therefore, if plants will exhibit sensitivity and/or will grow faster in presence of elevated amount of CO_2.

2. Materials and Methods

2.1. Germination Rate and Root/Stem Ratio Analysis

Germination rate was performed on seeds from two plants-species: rosemary (*Salvia rosmarinus*) and sage (*Salvia officinalis*).

The CO_2-enriched irrigation water (8g/L CO_2) was produced in the lab, mimicking the possible use of CO_2 by product from (waste)water treatment processes. Briefly, pressurized pure CO_2 gas (99.99% purity) was injected into tap water and the CO_2 concentration from the carbonated water was quantified in presence of sodium hydroxide. In total, 50 seeds for each plant were distributed on five transparent pots, 10 seeds per pots, filled with 50 g of gardening soil, purchased from SC Florisol (Botosani, Romania). Soil composition, according to manufacturer, had a minimum 70% organic substance of dry product, 6.5–7.0 pH, 60–70% humidity, 1.7% nitrogen, 0.21% phosphorus, 0.82% potassium, 34.48% organic matter, and 13.96% organic carbon. Seeded pots were irrigated every day with 1 mL per pot of plain water (control) or with CO_2-enriched water, then incubated in a HPP1060 climatic test chamber (Memmert, Eagle, WI, USA) having 15% of full light intensity (1500 lux), 25 °C temperature, and 50% humidity. Germination and plant development tests were monitored for up to 23 days. Plant–soil and microbial samples were collected at day 8 and 23, then seeds germination counts and root/stem ratios were analyzed as well as soil microbiological communities and physicochemical parameters.

2.2. Microbiological Analysis

The growth of aerobic or anaerobic bacterial populations was analyzed based on their total bacterial colonies count. Briefly, 10 g of soil sample was homogenized in distilled water in a ratio of 1:10. A volume of 1 mL from each dilution was inoculated into sterile Petri dishes with culture medium. Bacterial growth was carried out on aerobic or anaerobic conditions using Tryptic Soy Agar—Casein Soya Bean Dygest Agar (VWR, Leuven, Belgium) by being incubated at 22 °C for 72 h (aerobically) and 96 h (anaerobically). The results have been expressed as colony forming units per mL sample (CFU/mL).

2.3. Soil Measurements

Soil parameters (pH, electrical conductivity) were measured using the ORION STAR A329 portable multiparameter equipment from Thermo Scientific (Jakarta, Indonesia). Humidity was measured using KERN DAB 100-3 thermobalance manufactured by KERN & SOHN GmbH (Belingen, Germany).

2.4. Spectrum Analysis

The soil compounds were extracted with ethanol in a ratio of 1:3 (5 g soil:15 g ethanol), followed by 10 min centrifugation at 4600 rpm. The supernatant spectrum from 200 nm to 800 nm was acquired with a SHIMADZU spectrophotometer (Kyoto, Japan) [18].

3. Results

3.1. Plants Germination and Development

Plants seeds irrigated with CO_2-enriched water had a variable germination rate compared to their water irrigation control, which suggested a species specific CO_2 effect. Seeds from sage and rosemary plants, both belonging to the *Lamiaceae* family, showed very early, after 8 days, an opposite germination pattern.

Sage plants irrigated with CO_2-enriched water had up to 25% a germination boost compared to their control. In contrast, CO_2-enriched water inhibited around 30% rosemary seeds germination compared to control seeds (Figure 1). On a longer run, CO_2-enriched water proved to be critical for further rosemary plant development.

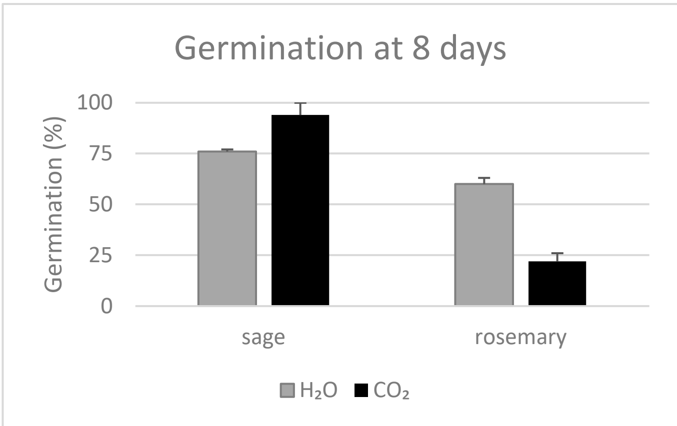


Figure 1. Germination rate after 8 days of sage and rosemary irrigated in presence or absence of CO_2-enriched water.

Germination results suggested that similar plants, belonging to the same family, had significantly different response to CO_2-enriched water. It is very important to establish a direct link between specific plants and a particular type of irrigation. Our results were consistent with previous reports showing a CO_2 effect based on plant species. An elevated CO_2 levels in soil could significantly impact the initial stages of germination and growth [19], due to plant elevated sensitivity to anaerobic conditions in its initial stages of growth [19]. Other data indicated that germination potential of four C4 plant species (corn, sugarcane, sorghum, common millet, or giant millet) remained unaffected until the CO_2 concentration reached 8% [20].

In general, higher CO_2 concentrations proved to negatively impact the plant development. A 20% CO_2 concentration in soil negatively impacted on winter bean crop, which failed to emerge, and 50–70% CO_2 soil concentration proved fatal for field beans [15]. There are also cases when CO_2 induced a beneficial acidification of soil. *Vitis vinifera* (L.), grown in Tempranillo vineyard calcareous soils, irrigated with acidified water, increased nutrient availability, which improved plant development, grape production, and quality. In addition, irrigation with acidified waters was an effective alternative to synthetic fertilizers and Fe chelates for managing iron chlorosis [21]. A further assessment of sage plant development irrigated or not with CO_2-enriched waters was carried out for up to 23 days. Most of the previous studies have primarily focused on germination based on a toxicity assessment [17,22] without taking into account the measurements of root and aerial part lengths. Roots play a critical role as they directly interact with the soil, absorbing and distributing water throughout the plant. The measurements of L. *sativa' s* root growth parameter from the current study was directly linked to seed germination potency [23], which greatly facilitates the collection and analysis of research outcomes.

Results showed that sage root development were more robust when irrigated with CO_2-enriched water for a shorter period of time. Roots/stem ratio at 8 days was 4.81 for sage watered with CO_2-enriched water compared to a ratio of 3.74 for plants irrigated with plain water (Table 1). The roots/stem ratio became almost the same after 23 days regardless of irrigation type, with or without CO_2 added, but a more robust roots system seemed to support the overall plant growth.

Table 1. Plant development in presence or absence of CO_2-enriched water.

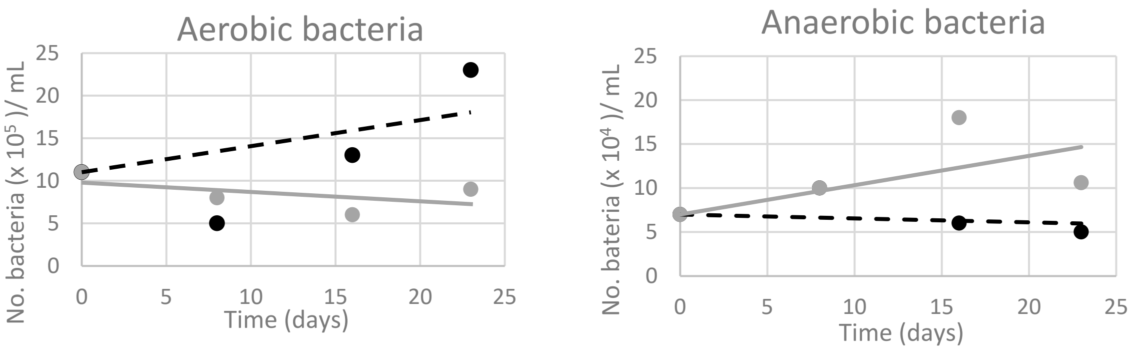
	Roots/Stem Ratio		**Plant Length (cm)**	
	8 days	**23 days**	**8 days**	**23 days**
H_2O	3.74	0.54	7.23	13.24
CO_2	4.81	0.58	7.48	15.75

STD +/− 5%.

On short terms, sage plants irrigated with CO_2-enriched water grew up to 7.48 cm in 8 days, compared to control plants, which grew up to 7.23 cm. For longer term, the overall plants size irrigated with CO_2-enriched water grew bigger (average of 15.75 cm) compared to control plants (average of 13.24 cm) (Table 1). The results at 23 days showed a higher sage overall growth, up to 20%, when irrigated with CO_2-enriched water compared to control samples. It was reported that soils irrigated with CO_2-enriched water were more permeable, allowing a better aeration, subsequently promoting better germination and faster root growth rate [24]. A well-developed plant further increase its growth due to a high CO_2 concentrations which had a stimulating effect on stomatal and whole plant development [25]. The observations suggested that plants have responded differently to CO_2 input depending on their species and specific adaptation mechanisms to these new environmental conditions. The CO_2 impact on soil physical properties (Table 2) and microorganism from soil (Figure 2) most likely played a significant effect on plant germination and development.

Table 2. Physical parameter of sage planted soil irrigated or not with CO_2-enriched water.

	pH		**Electrical Conductivity (µS/cm)**		**Humidity**	
	8 days	**23 days**	**8 days**	**23 days**	**8 days**	**23 days**
H_2O	7.41	7.65	878	806	52.4	60.6
CO_2	7.31	7.35	935	884	54.9	58.2



(**A**) (**B**)

Figure 2. The aerobic (**A**) and anaerobic (**B**) bacterial population dynamics from soils irrigated with CO_2-enriched water (gray continuous line) and plain water (black dotted line). The dashed and dotted lines are linear fittings of experimental data.

3.2. Physical Analyses of Soil

Physical parameters (pH, conductivity, and humidity) of sage planted soil were monitored up to 23 days, during plant germination and development (Table 2). The CO_2-enriched water irrigation kept an acidified soil, enhancing the pH gap to 0.2 pH units, at 23 days, between water and CO_2-enriched water irrigation (Table 2).

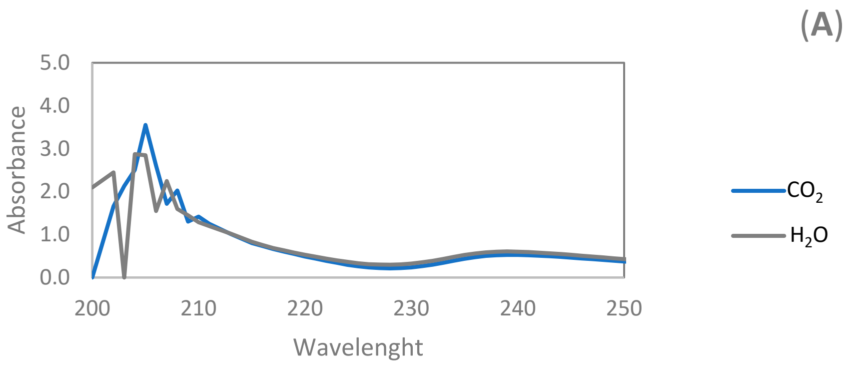
At the same time, CO_2-enriched water irrigation enhanced soil conductivity compared to plain water irrigation. Humidity monitoring at 25 °C showed a higher humidity for CO_2-enriched water irrigated soil after 8 day, but the humidity pattern was reversed for longer periods of time, up to 23 days (Table 2).

3.3. Microbiological Soil Analyses

The plant germination and development could also be influenced by soil microbes such as bacteria. Symbiotic relationships between bacteria and plants, especially at the roots level, have been reported to be beneficial for plant development and growth [26]. Compounds with nitrogen are very important for plant development and, therefore, the nitrification and denitrification balance plays an important role in aerobic and anaerobic processes. These processes have been dependent on aerobic and anaerobic bacterial population which are sensitive to environmental changes, such as CO_2 amounts. The dynamics of aerobic and anaerobic bacteria from soil were analyzed in sage planted soil irrigated in presence or absence of CO_2-enriched water (Figure 2).

CO_2 had a clear influence on soil microbial dynamics by decreasing the aerobic bacterial population over time. Aerobic bacterial populations were thriving in sage planted soils irrigated with plain water (Figure 2A). An opposite bacterial growth pattern was observed on anaerobic bacteria from soil irrigated with CO_2-enriched water, which showed a significant growth compared to anaerobic bacteria from control soil (irrigated only with plain water) (Figure 2B).

Changes in plant germination and development pattern as well as in bacterial populations had an impact on chemical composition of soils due to specific metabolic pathways of anaerobic or aerobic bacteria. Sage planted soil samples from plant germination and development irrigated in presence or absence of CO_2-enriched water were spectrophotometrically analyzed. The spectral analyses of soil at day 8 showed a high and relatively constant peak between 205 and 230 nm, suggesting a significant presence of dissolved organic matter and nitrogenous compounds such as nitrates, nitrites, proteins, organic acids (Figure 3A).



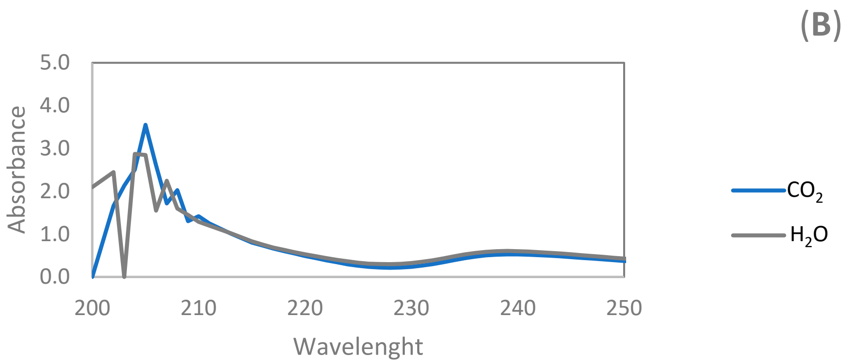


Figure 3. Spectrophotometric analyses of soil irrigated or not (control) with CO_2-enriched water for 8 days (**A**) or 23 days (**B**).

There were some differences between samples irrigated with CO_2-enriched water and plain water suggesting a quick impact of CO_2. At around 205 nm, nitrogenous compounds concentration was decreased in soils irrigated with CO_2-enriched water compared to plain water irrigation. The organic matter spectral pattern around 214 was reversed when soils irrigated with CO_2-enriched water had higher compounds concentrations compared to plain water irrigation. Aerobic bacteria may have played a key role in decomposing organic matter, utilizing dissolved organic carbon as anaerobic bacteria in nitrogenous compounds' mineralization processes.

Spectral data confirm that nitrogenous compounds (e.g., nitrates, nitrites, proteins) persist over time, with an absorption peak at around 205 nm, meaning the soil had not yet exhausted its nutritional capacity [27]. The spectral and microbiological data suggest a strong link between microbial activity and organic matter degradation, nitrogen cycling, and soil adaptation to CO_2. CO_2 may accelerate microbial activity and mineralization, leading to a faster release of nutrients. This suggests that anaerobic nitrogen-transforming bacteria, such as denitrifiers (*Paracoccus* sp., *Pseudomonas* sp.) and nitrate reducers (*Clostridium* sp., *Desulfovibrio* sp.), could be responsible for maintaining nitrogen compound levels despite organic matter reduction [28].

Overall, the correlation between spectral and microbiological data showed that microbial activity plays a crucial role in soil evolution. Aerobic bacteria contribute to organic matter degradation and nutrient cycling, but anaerobic bacteria help maintain nitrogenous compounds through denitrification and other nitrogen transformations.

4. Conclusions

This study demonstrated that CO_2-enriched water, as a (waste)water treatment process byproduct, could be used in agriculture to mitigate greenhouse gasses production, according to circular economy principles. CO_2-enriched water induced a plant species-specific responses for germination and early-stage development, playing a crucial role in plant adaptation to elevated CO_2 levels. Rosemary exhibited adverse effects, such as reduced germination, root development, and decreased total biomass, but sage showed an opposite trend, benefiting from CO_2 exposure. It seemed that a high soil CO_2, not low O_2 or pH [16], was responsible to modulate different physiological and biochemical mechanisms triggered by plants to cope with environmental stressors [29]. This suggested that early-stage exposure to high CO_2 could have long-term consequences on plant resilience and productivity [30,31]. In addition to the direct effects of CO_2 on plant physiology, this study also revealed changes in soil physicochemical parameters and soil microbial population and activity, indicating that CO_2 addition altered the soil nutrient cycle and microbial community dynamics. These shifts could have further implications for plant development, as soil–plant–microbe interactions have been essential for nutrient uptake and overall ecosystem stability. Overall, CO_2 profoundly impacted root and stem development during the initial stages of plant growth, but it also modified the bacterial population dynamics in soil, which could induce more changes in plant development. This research was highly relevant in the context of a climate change perspective, where plants have been increasingly exposed to CO_2 fluctuating levels and other environmental stressors. Understanding how plants responded to these challenges was critical in developing strategies to link raised CO_2 levels with enhance crop resilience, improve agricultural sustainability, and maintain an ecosystem balance. Moreover, the potential application of CO_2-enriched water for irrigation opened new avenues for optimizing water use efficiency and modulating plant stress responses. Overall, this study provided valuable insights into the complex interactions between plants, soil microbiota, and elevated CO_2 levels, emphasizing the importance of species-specific adaptation strategies. Continued research in this field will be essential for improving circular economy where greenhouse gasses byproducts from various (waste)water technological processes could be turned into solution for a sustainable agricultural management and environment, enhancing climate change resilience.

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Data Availability Statement: The data presented in this study are openly available in repository SSRN: The effects of CO_2-enriched water irrigation on agricultural crop development by Laura FEODOR, https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5195684 (accessed on 27 March 2025).

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Conflicts of Interest: Authors Indraneel Sen and Yasmina Dimitrova were employed by Wasabi Innovations Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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